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Fusion of the *EWS1* and *WT1* Genes as a Result of the t(11;22)(p13;q12) Translocation in Desmoplastic Small Round Cell Tumors

Laura E. Benjamin, PhD, William J. Fredericks, PhD, Frederic G. Barr, MD, PhD, and Frank J. Rauscher III, PhD

The isolation and molecular analysis of genes which cause and/or predispose to Wilms' tumor have yielded fascinating insights into the role of tissue-specific gene regulation in both development and disease processes. Analysis of the *WT1* transcription factor has clearly established its role in Wilms' tumorigenesis and a broader role in both urogenital organogenesis and mesenchymal cell differentiation events. Clearly, loss of function mutations in *WT1* is correlated with aberrant function as a regulator of gene expression, ultimately resulting in neoplastic transformation in the developing kidney. A question we have pursued is whether alterations of *WT1* structure and/or function can be associated with other types of malignancies, possibly reflecting *WT1*'s broader role in mesenchymal differentiation. To this end, we have analyzed a rare solid tumor designated *I*ntra-*A*bdominal *D*esmoplastic *S*mall *R*ound *C*ell *S*arcoma (IADSRCT) which often displays a recurrent chromosomal translocation t(11;22)(p13;q12) involving the *WT1* genomic locus. We have shown that the *EWS1* gene from chromosome 22q12 is fused to the *WT1* gene in IADSRCT and that a fusion protein is produced which functions as a potent activator of transcription. Our results suggest that *WT1* has sustained a gain-of-function alteration as a result of this fusion and that the fusion gene functions as a dominant oncogene in this disease. Thus, the *WT1* locus may be the target for both gain- and loss-of-function mutations resulting in different disease outcomes. A summary of our ongoing analysis of the *EWS-WT1* fusion gene is presented.

Key words: Ewing's sarcoma, Wilms' tumor, *WT1*, chromosomal translocation, molecular analysis, urogenital organogenesis

WT1 AND WILMS' TUMORIGENESIS

A summary of important structure/function characteristics for *WT1* is shown in Table 1 [compiled from the review articles 1, 2, 3]. The *WT1* genomic locus occupies ~50 kb on chromosome 11p13 and produces a transcript of ~3.5 kb. As expected for a tumor suppressor or recessive oncogene, *WT1* is mutated or deleted in Wilms' tumors.

In order to understand the role of *WT1* in the functioning of the urogenital organs and in the genesis of Wilms' tumor, researchers have studied both the regulation of the *WT1* gene, and the function of the *WT1* protein. Efforts to understand the regulation of *WT1* began with a description of its RNA expression patterns, and continued with analysis of the protein expression patterns. These data provide the foundation on which *WT1's* proposed role in development has been based.

The RNA expression pattern of the *WT1* gene correlates with some of the tissues affected by mutations in *WT1*. A ~3.5 kb *WT1* messenger RNA transcript was detected in human fetal kidney, spleen, testis, and ovary, and brain by Northern blot analysis [4]. Analysis of *WT1* transcripts by in situ hybridization in a 6-week human fetus showed expression is high in the gonadal mesenchyme cells that differentiate into the sex cord epithelium, and in the mesenchymal cells that differentiate into mesothelium. Analysis of 6- and 18-week human fetal kidneys showed *WT1* RNA levels increasing in the metanephric mesenchyme of the developing nephron as induction from the ureteric bud proceeds [5]. Additionally, levels of *WT1* message correlated with the state of differentiation in Wilms' tumors: high levels were seen in tumors with a higher percentage of undifferentiated mesenchyme (blastemal) cells [6]. Analysis of the murine *WT1* showed similar patterns of expression in the embryo and developing fetus. In adult tissues, expression is restricted to renal podocytes, Sertoli cells of the testis, granulosa cells of the ovary, mesothelial linings of organs, and the decidual cells of the uterus [7–9]. Together the expression data on *WT1* suggested that *WT1* expression correlates with the transition from mesenchyme to epithelium.

WT1 encodes a transcription factor containing four zinc fingers of the C_2H_2 class in the COOH-terminus and

From the Wistar Institute (L.E.B., W.J.F., F.J.R.) and Department of Pathology and Laboratory Medicine (F.G.B.), Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania.

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Address reprint requests to F.J. Rauscher, III, The Wistar Institute, 3601 Spruce Street, Rm. 123, Philadelphia, PA 19104.

TABLE I. An Overview of the *WT1* Gene

Location	Chromosome 11p13
Size	~50 kb genomic locus
Structure	10 exons, 2 alternative splices
mRNA	~3.5 kb
Expression patterns	
Embryonic	Kidney (condensing metanephric blastema and podocytes)
	Mesothelial lining (all organs)
	Gonadal ridge mesothelium
	Spleen
	Brain (area postrema)
	Spinal cord (ventral horn motor neurons)
Adult	Kidney (glomerular epithelium)
	Ovary (granulosa cells)
	Testis (sertoli cells)
	Uterus (decidual cells)
Protein product	52-54 kDa, nuclear protein
Structural motifs	4 Cys_2-His_2 zinc fingers, glutamine-proline-glycine-rich transcriptional regulation domain
Interacting proteins	p53
DNA-binding site	*EGR* consensus sequence: 5′-GGAGCGGGGGCG-3′
Target genes	IGF-II, IGF-II receptor, EGR-1, Pax-2, PDGF-A, CSF-1, TGF-beta1
Diseases associated with	Wilms' tumor, DDS, mesothelioma, IADSRCT

an amino terminus rich in proline, glutamine, serine, and glycine [10–12]. Fingers 2–4 are 61% homologous to the zinc fingers of *EGR-1* with identity at the DNA contact residues defined by *EGR-1* crystallographic studies [13]. The *WT1* (−KTS) isoform (which contains a three amino acid insertion in the DNA-binding domain), but not *WT1* (+KTS), is able to bind to the *EGR*-consensus sequence GCG/TGGGGCG [14]. Targets for the *WT1* (+KTS) isoform have been identified, but these were also found to bind *WT1* (−KTS) more efficiently [15]. One hypothesis is that *WT1* (+KTS) does not function in transcriptional regulation, and either has completely different functions or modulates the functions of the other isoforms in some fashion. Evidence supporting the importance of the (+KTS) isoform is obtained from several Denys-Drash syndrome (DDS) mutations which do not inactivate the function of the resulting *WT1* protein, but simply eliminate the insertion of the KTS splice. In these patients, the only detectable *WT1* alteration is in the relative ratios of their *WT1* isoforms [2]. Efforts to identify the functions of individual splice variants are ongoing, however recent studies have implicated the +KTS variant in RNA splicing due to colocalization with splicing structures in the nucleus [16]. It is possible that *WT1* may play a role in both DNA and RNA binding. The *EGR* consensus sequence contains nine nucleotides (5′-GCGGGGGCG-3′) which bind the three zinc fingers (fingers 2–4), which show the greatest homology to *EGR-1*. Thus, *WT1* is a sequence-specific DNA-binding protein which binds the *EGR*-consensus sequence and variants thereof.

THE *WT1* PROTEIN AND TRANSCRIPTIONAL REPRESSION

A second biochemical function of the *WT1* protein is transcriptional regulation. While the zinc-finger domain mediates DNA binding, the amino-terminal domain contains transcriptional repressing function. Deletion analyses of *WT1* demonstrated that DNA binding through the zinc fingers as well as sequences in the amino-terminus are necessary for transcriptional regulation [17,18]. To understand how *WT1* mutation leads to Wilms' tumor, it is necessary to define the set of genes regulated by *WT1*. The *EGR*-consensus sequence is found in the promoters of *EGR-1* as well as in several other promoters. *WT1* has been shown to repress transcription from several of these promoters in cotransfection assays. These include *EGR-1* [17], *PDGFA* [19,20], *CSF-1* [21], *IGF-II* [22], *IGF-IR* [23], and *Pax-2* [24]. In many of these studies, *WT1* was shown to physically bind these promoter sequences and protect specific sequences from DNAse digestion. Additionally, *WT1* has been shown to repress transcription from its own promoter, providing one mechanism for autoregulation of *WT1* expression [25]. Thus, *WT1* was shown to bind DNA sequence specifically, and regulate transcription of potential target genes which contain *EGR*-like sequences in their promoters.

WT1 MUTATIONS IN DDS

The most compelling evidence correlating *WT1* mutations and Wilms' tumor comes from patients with the congenital condition DDS. In addition to intersex disor-

ders and nephropathy, these patients have an increased chance of developing Wilms' tumor [2]. In DDS patients, >95% harbor constitutional missense mutations or very small deletions in the zinc-finger region of *WT1*. The most common mutation (56%) in DDS is a missense mutation in amino acid 394 of the third zinc finger. Hypotheses regarding the severity of the DDS phenotype due to heterozygous mutations invoke a dominant-negative action of these mutations. Nonetheless, deletion of both alleles of *WT1* appears to be required for tumor formation in these patients.

WT1 MUTATION IN THE ETIOLOGY OF OTHER TUMORS

An example of one tumor related to *WT1* developmental expression is mesothelioma. *WT1* is expressed in the mesothelial linings of the developing peritoneal organs. One peritoneal mesothelioma has been reported with a heterozygous point mutation in the regulatory domain of the *WT1* protein. In this tumor it appeared that this *WT1* allele encoded a protein which activated transcription [26]. This mutation was found in 1 of 78 tumors examined, and may not represent a common mechanism in the etiology of mesothelioma. However, it may be revealing that *WT1* misregulation or malfunction is deleterious to mesothelial cells, and other ways to deregulate *WT1* function may be more commonly associated with mesothelioma.

Thus, the picture of *WT1* which has emerged suggests a developmentally regulated transcription factor which may have a broad role in mesothelial or mesenchymal cell differentiation. In view of this, we began to examine *WT1* in tumors which appear to arise from mesothelial tissues. One such tumor is a small round cell sarcoma designated intra-abdominal desmoplastic small round cell tumor (IADSRCT). The biologic and molecular genetic characteristics of IADSRCT are described below.

IADSRCT

IADSRCT is a very aggressive, rare tumor that occurs most frequently in adolescent males and is located almost exclusively in the abdomen [27]. Patients at presentation often have tumor involvement of many abdominal organs as well as the serosal lining of the abdomen. Because of the widespread location for this tumor at presentation, the primary site of tumor development and of the target cell for oncogenic transformation has remained elusive. Histopathology shows a "nested" pattern of tumor cell growth containing islands of densely packed small round cells surrounded by desmoplastic stroma. Immunohistochemical analysis demonstrates that IADSRCTs often coexpress epithelial, mesenchymal, and neural markers [27]. Together, these findings have led pathologists to consider other designations for this tumor based on this apparently primitive "blastomatous" cellular phenotype, including mesothelioblastoma, peritoneal blastoma, or extrarenal Wilms' tumor. This last designation stems in part from similarities among DSRCTs and "classic" triphasic Wilms' tumors which also contain epithelial, mesenchymal, and stromal cell elements.

The IADSRCT was recently described as an independent member of the family of small round cell tumors. An extensive histopathological analysis of 22 IADSRCT cases and a literature survey of 35 more led Ordonez et al. [27] to concur with Gonzalez-Crussi et al [28] that the tumor arises from the serosal lining of the abdomen. They suggest that the subserosal mesenchyme has the morphological variations to account for the divergent differentiations of IADSRCT [27]. The subserosal mesenchyme is a unique tissue layer referred to as mesothelium since it has properties of both mesoderm and epithelium. This tissue layer lines the pleural, pericardial, and peritoneal cavities and is a site of *WT1* expression.

Most intriguing were cytogenetics reported in a number of IADSRCTs by independent investigators who identified a recurring translocation t(11;22)(p13;q12) [29–31]. The breakpoints in this rearrangement suggested the possibility of *WT1* (11p13) and *EWS1* (22q12) involvement. This, and the knowledge that *WT1* is expressed in the mesothelium, led us to hypothesize that the *EWS1* gene had become fused to *WT1* in IADSRCT.

The *EWS1* gene was originally cloned as a result of intense molecular characterization of the chromsomal translocation t(11;22)(q13;q12) [reviewed in 32]. This cytogenetically defined translocation is often found in the small round cell tumor Ewing's sarcoma. Cloning of the breakpoint revealed that *EWS1*, a putative RNA binding protein (Fig. 1), is fused in-frame to the DNA-binding domains of multiple transcription factors depending on tumor type. The NH_2-terminal domain (abbreviated "NTD") of *EWS1* is a highly potent transcriptional activation domain which is fused to a variety (Fig. 1) of ETS-type or b-zip-type DNA-binding domains. The resulting chimeric transcription factors are both potent activators of transcription and dominantly acting oncogenes.

Thus, the cytogenetic reports of a recurrent t(11;22)(p13;q12) in IADSRCT strongly implicated the *EWS1* and *WT1* genes. Furthermore, based upon the fact that the NTD of *EWS1* is always fused to a DNA-binding domain, we could immediately focus on the zinc-finger region of *WT1*.

FUSION OF *EWS1* TO THE *WT1* ZINC-FINGER REGION

We designed a polymerase chain reaction (PCR)-based assay using primers specific for the *EWS1-NTD* and the zinc-finger region of *WT1*. Reverse-transcription and PCR from IADSRCT-derived RNA revealed a fusion

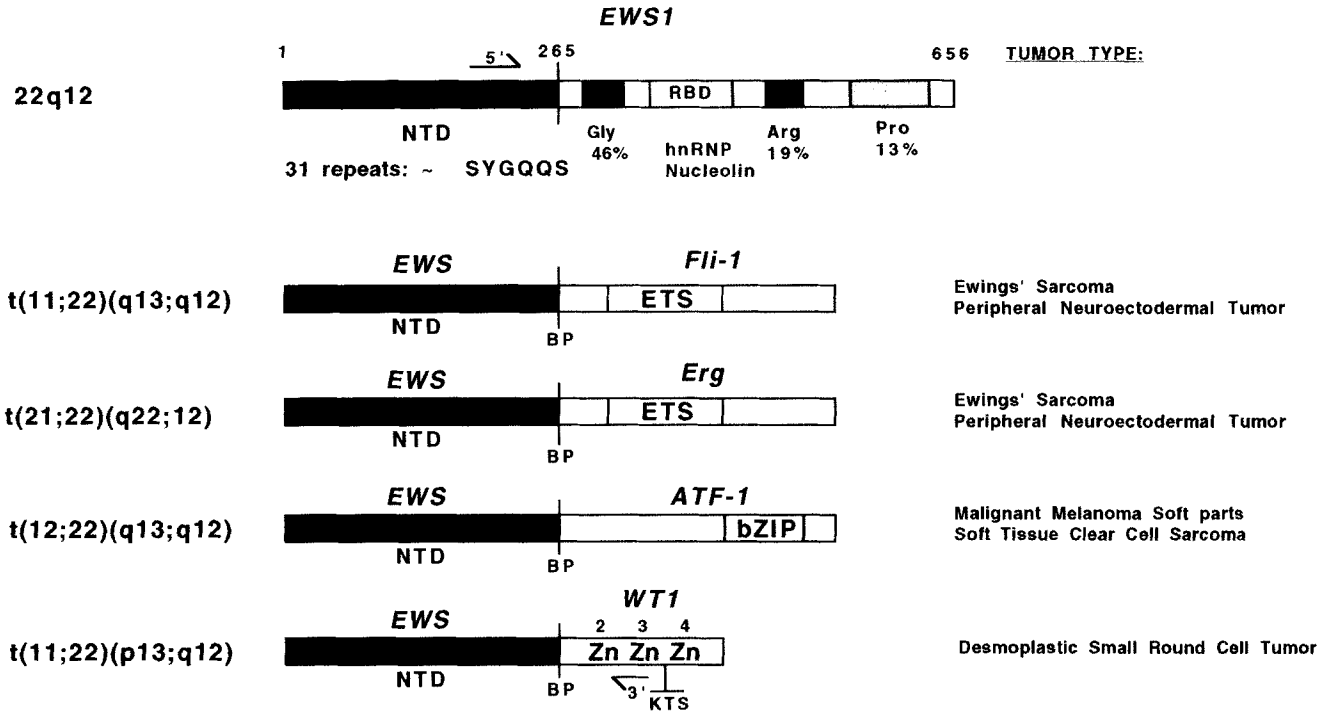

Fig. 1. An overview of the Ewing's sarcoma gene (*EWS1*) is shown. The structural characteristics of the *EWS1* protein are highlighted. As a result of specific chromosomal translocation, the NTD of *EWS1* is fused to the indicated DNA-binding domains. BP = breakpoint.

transcript between *EWS1* and *WT1* in 11 of 12 tumor specimens [33 and unpublished data, FG Barr]. The fusion involved exon 7 of *EWS1* and exon 8 (finger 2) of *WT1*. All of the genomic breakpoints are intronic and variable [34 and unpublished data, FG Barr]. However, proper splicing creates identical fusion junctions in the transcribed RNA. The predicted *EWS1-WT1* fusion protein is in-frame and suggests that the potent NTD activation domain has replaced the repression domain of *WT1* (Fig. 2). In addition, loss of zinc finger 1 in the fusion would be expected to alter the DNA-binding properties of the fusion protein.

Analysis of DNA-binding using zinc-finger recombinant proteins illustrated that the splice-generated isoform *EWS-WT1 (+KTS)* had reduced binding abilities to *EGR*-based consensus target sequences compared to *EWS-WT1 (−KTS)*. Interestingly, *EWS-WT1 (−KTS)* and *WT1* bound similar DNA targets, but *EWS-WT1* did so with increased affinity. The similarity between the DNA-binding domains of *EGR-1* and *EWS-WT1 (−KTS)* suggested that t(11;22)(p13;q12) had created an *EGR*-1-like transcriptional activator. However, a comparison of *EWS-WT1 (−KTS)* and *EGR-1* revealed differences in their binding preferences. We have shown that the *EWS-WT1 (−KTS)* fusion protein has the ability to activate transcription from target promoters containing *EGR*-consensus-like sequences in NIH 3T3 cells and human mesothelial cells. Finally, we have demonstrated the oncogenic poten-

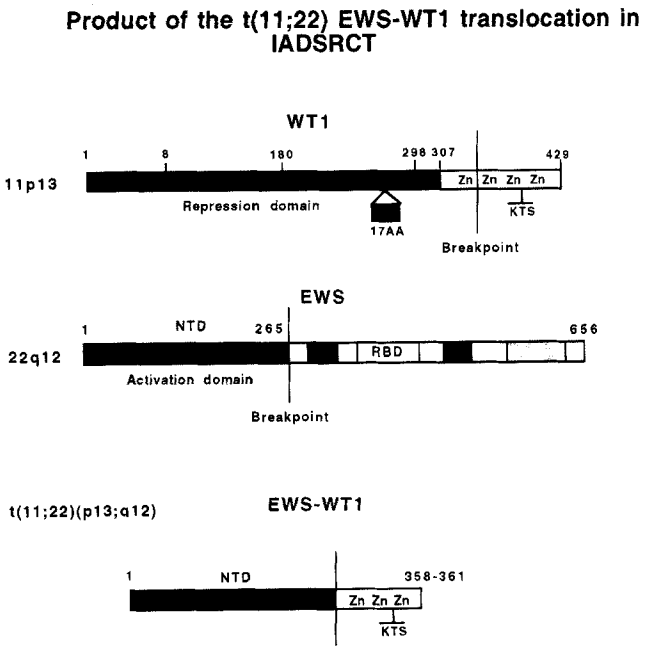

Fig. 2. The *EWS1* and *WT1* genes fuse to form *EWS-WT1*. The structures of the resulting chimeric and wild-type *EWS1* and *WT1* proteins are shown.

tial of *EWS-WT1* using retroviral infection of NIH 3T3 cells followed by growth in soft agar.

CONCLUSIONS

We have identified and initiated the molecular characterization of a novel nonrandom chromosomal translocation involving the *EWS1* and *WT1* genes. The fusion of these genes alters the functional properties of both proteins. It appears that *WT1*, initially identified as a recessive oncogene, sustains a gain-of-function alteration (enhanced transcriptional activation potential as a result of fusion to the NTD of *EWS1*). By analogy to other transcription factor fusions involving *EWS1*, it is likely that the *EWS-WT1* fusion functions as a dominant oncogene in the etiology of IADSRCT. It will be interesting to determine if downstream target genes, normally repressed by wild-type *WT1* in mesothelial cells are activated and deregulated by the *EWS-WT1* fusion protein.

REFERENCES

1. Rauscher FJ III: The *WT1* Wilms' tumor gene product: A developmentally regulated transcription factor in the kidney that functions as a tumor suppressor. FASEB J 7:896–903, 1993.
2. Coppes MJ, Campbell CE, Williams BRG: The role of *WT1* in Wilms' tumorigenesis. FASEB J 7:886–895, 1993.
3. Hastie ND: The genetics of Wilms' tumor—a case of disrupted development. Annu Rev Genet 28:523–528, 1994.
4. Huang A, Campbell CE, Bonetta L, McAndrews-Hill MS, Chilton-MacNeill S, Coppes MJ, Law DJ, Feinberg AP, Yeger H: Tissue, developmental, and tumor-specific expression of divergent transcripts in Wilms' tumor. Science 250:991–994, 1990.
5. Pritchard JK, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, Bard J, Buckler A, Pelletier J, Housman D, VanHeyningen V, Hastie N: The candidate Wilms' tumour gene is involved in genitourinary development. Nature 346:194–197, 1990.
6. Pritchard JK, Fleming S: Cell types expressing the Wilms' tumour gene *(WT1)* in Wilms' tumours: Implications for tumour histogenesis. Oncogene 6:2211–2220, 1991.
7. Armstrong JF, Pritchard JK, Bickmore WA, Hastie ND, Bard JB: The expression of the Wilms' tumour gene, *WT1*, in the developing mammalian embryo. Mech Dev 40:85–97, 1993.
8. Buckler AJ, Pelletier J, Haber DA, Glaser T, Housman DE: Isolation, characterization, and expression of the murine Wilms' tumor gene *(WT1)* during kidney development. Mol Cell Biol 11: 1707–1712, 1991.
9. Rackley RR, Flenniken AM, Kuriyan NP, Kessler PM, Stoler MH, Williams BR: Expression of the Wilms' tumor suppressor gene *WT1* during mouse embryogenesis. Cell Growth Differ 4:1023–1031, 1993.
10. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber D, Rose EA, Kral A, Yeger H, Lewis WH, Jones C, Housman DE: Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 60:509–520, 1990.
11. Gessler M, Poustka A, Cavanee W, Neve RL, Orkin SH, Bruns GA: Homozygous deletion in Wilms' tumours of a zinc finger gene identified by chromosome jumping. Nature 343:774–778, 1990.
12. Morris JF, Madden SL, Tournay OE, Cook DM, Sukhatme VP, Rauscher III FJ: Characterization of the zinc finger protein encoded by the WT1 Wilms' tumor locus. Oncogene 6:2339–2348, 1991.
13. Pavletich NP, Pabo CO: Zinc finger DNA recognition: Crystal structure of a zif268-DNA complex at 2.1A. Science 252:809–817, 1991.
14. Rauscher III FJ, Morris JF, Tournay OE, Cook DM, Curran T: Binding of the Wilms' tumor locus zinc finger protein to the *EGR-1* consensus sequence. Science 250:1259–1262, 1990.
15. Bickmore WA, Oghene K, Little MH, Seawright A, Van HV, Hastie ND: Modulation of DNA binding specificity by alternative splicing of the Wilms' tumor *WT1* gene transcript. Science 257:235–237, 1992.
16. Larsson SH, Charlieu J-P, Miyagawa K, Engelkamp D, Rassoulzadegan M, Ross A, Cuzin F, van Heyningen V, Hastie ND: Subnuclear localization of *WT1* in splicing or transcription factor domains is regulated by alternative splicing. Cell 81:391–401, 1995.
17. Madden SL, Cook DM, Morris JF, Gashler A, Sukhatme VP, Rauscher III FJ: Transcriptional repression mediated by the *WT1* Wilms' tumor gene product. Science 253:1550–1553, 1991.
18. Madden SL, Cook DM, Rauscher III FJ: A structure-function analysis of transcriptional repression mediated by the *WT1*, Wilms' tumor suppressor protein. Oncogene 8:1713–1720, 1993.
19. Gashler AL, Bonthron DT, Madden SL, Rauscher III FJ, Collins, Sukhatme VP: Human platelet-derived growth factor A chain is transcriptionally repressed by the Wilms' tumor suppressor *WT1*. Proc Natl Acad Sci USA 89:10984–10988, 1992.
20. Wang ZY, Madden SL, Deuel TF, Rauscher III FJ: The Wilms' tumor gene product, *WT1*, represses transcription of the platelet-derived growth factor A chain gene. J Biol Chem 267:21999–22002, 1992.
21. Harrington MA, Konicek B, Song A, Xia XL, Fredericks WF, Rauscher III FJ: Inhibition of colony-stimulating factor-1 promoter activity by the product of the Wilms' tumor locus. J Biol Chem 268:21271–21275, 1993.
22. Drummond IA, Madden SL, Rohwer NP, Bell GI, Sukhatme VP, Rauscher III FJ: Repression of the insulin-like growth factor II gene by the Wilms' tumor suppressor *WT1*. Science 257:674–678, 1992.
23. Werner H, Roberts CJ, Le RD: The regulation of *IGF-1* receptor gene expression by positive and negative zinc-finger transcription factors. Adv Exp Med Biol 343:91–103, 1993.
24. Ryan G, Steele-Perkins V, Morris JF, Rauscher III FJ, Dressler GR: Repression of Pax-2 by *WT1* during normal kidney development. Development 121:867–875, 1995.
25. Rupprecht HD, Drummond IA, Madden SL, Rauscher III FJ, Sukhatme VP: The Wilms' tumor suppressor gene *WT1* is negatively autoregulated. J Biol Chem 269:6198–6206, 1994.
26. Park S, Schalling M, Bernard A, Maheswaran S, Shipley GC, Roberts D, Fletcher J, Shipman R, Rheinwald J, Demetri G, Griffen J, Minden M, Housman DE, Haber D: The Wilms' tumour gene *WT1* is expressed in murine mesoderm-derived tissues and mutated in a human mesothelioma. Nat Genet 4:415–420, 1993.
27. Ordonez NG, El-Nagger AK, Ro JY, Silva EG, Mackay B: Intraabdominal desmoplastic small cell tumor: A light microscopic immunocytochemical, ultrastructural, and flow cytometric study. Hum Pathol 24:850–865, 1993.
28. Gonzalez-Crussi F, Crawford SE, Sun CJ: Intraabdominal desmoplastic small cell tumors with divergent differentiation. Am J Surg Pathol 14:633–642, 1990.
29. Beigel JA, Conard K, Brooks JJ: Translocation (11;22)(p14;q12): Primary change in intra-abdominal desmoplastic small round cell tumor. Genes Chromosom Cancer 7:119–121, 1993.
30. Rodriguez E, Sreekantaiah C, Gerald W, Reuter VE, Motzer RJ,

Chaganti RS: A recurring translocation, t(11;22)(p13;q11.2), characterizes intraabdominal desmoplastic small round cell tumors. Cancer Genet Cytogenet 69:17–21, 1993.
31. Shen WP, Towne B, Zadeh TM: Cytogenetic abnormalities in an intra-abdominal desmoplastic small cell tumor. Cancer Genet Cytogenet 64:189–191, 1992.
32. Delattre O, Zucman J, Medot T, Garau XS, Zucker JM, Lenoir GM, Ambros PF, Sheer D, Turc-Carel C, Triche TJ, Aurias A, Thomas G: The Ewing family of tumors—A subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med 331:294–299, 1994.
33. Rauscher III FJ, Benjamin LE, Fredericks WJ, Morris JF: Novel oncogenic mutations in the *WT1* Wilms' tumor suppressor gene: A t(11;22) fuses the Ewing's sarcoma gene, *EWS1*, to *WT1* in desmoplastic small round cell tumor. Cold Spring Harbor Symp Quant Biol LIX:137–146, 1995.
34. Ladanyi M, Gerald W: Fusion of the *EWS* and *WT1* genes in the desmoplastic small round cell tumor. Cancer Res 54:2837–2840, 1994.

COMMENTARY

Until recently, most investigators only recognized the involvement of *WT1* as a tumor-suppressor gene in the development of Wilms' tumor, DDS, and possibly certain mesotheliomas. Benjamin et al. describe an exciting new property of *WT1*. They demonstrate that after a translocation involving 22q12 (harboring the Ewing sarcoma gene *EWS1*) and 11p13 (harboring *WT1*) a novel chimeric protein plays a role in the development of desmoplastic small round cell tumors. In other words, in this context, *WT1* has become part of a dominant oncogene. This observation is remarkable and sheds a truly novel light on the putative properties of the *WT1* gene.